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Knockdown of cathepsin Z by siRNAs is associated with reduced cell invasion in osteosarcoma cells

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Abstract

Background: *Osteosarcoma is the most common primary bone cancer. The proteolytic enzyme, cathepsin Z, which has been associated with cancers, is produced in bone-marrow-derived osteoblast cells and in cultured osteoblast-like osteosarcoma cells. The aim of the present experiments was to examine the expression and function of cathepsin Z protein in cultured osteosarcoma cells.*

Methods: *The presence of cathepsin Z protein and estrogen receptor α in three cultured osteosarcoma cell lines, MG-63, TE-85 and SAOS-2 were examined using Western blotting. Specific SiRNAs targeting cathepsin Z mRNA were used to knock-down cathepsin Z protein in cultured osteosarcoma cells.*

Results: *The results showed that cathepsin Z was present abundantly in osteosarcoma cells, MG-63, that represent an early osteoblast phenotype, and was also present in TE-85 cells. Cathepsin Z was below the level of detection in SAOS-2 cells, which represent more mature osteoblast cells. Knock-down of cathepsin Z protein by specifically targeted siRNAs in the MG-63 cells had only a small effect on cell migration in a scratch wound assay, but dramatically reduced the chemotactic migration/invasion of these cells through a Matrigel matrix.*

Conclusions: *This is the first time that it has been demonstrated that cathepsin Z might play an important role in the invasive properties of less well differentiated osteosarcoma cells and indicate that cathepsin Z might contribute to osteosarcoma progression.*

Keywords: *cathepsin Z, SiRNAs, osteosarcoma cells, estrogen receptor α, cell invasion*

Abbreviations: *ANOVA: Analysis of variance; DMEM: Dulbecco's modified Eagles' medium; DMSO: Dimethyl sulfoxide; DPBS: Dulbecco's phosphate buffered saline; FBS: Foetal bovine serum; MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; RIPA: radio immunoprecipitation assay; RT-PCR: Reverse transcription polymerase chain reaction; SiRNA: Small-interfering-RNA sequence; TBST: Tris-buffered saline containing Tween 20.*

Background

Osteosarcoma is the most common bone cancer [1-3]. Osteosarcomas are thought to be derived from osteoblast cells [4] and some cultured osteosarcoma cells, MG-63, TE-85 and SAOS 2 exhibit increasing degrees of osteoblast differentiation [5,6]. The least differentiated cell line, MG-63 has been reported to produce the protease cathepsin Z [7].

Cathepsin Z, also known as cathepsin X or P [8] is produced as a precursor, pro-cathepsin of molecular mass of about 40 kDa [7], which is processed to a proteolytically-active mature form of molecular mass 34 kDa [8]. The pro-form, but not the mature form, contains an RGD integrin-binding region, which is thought to direct cathepsin Z activity outside the cell via interaction with integrins [9]. Cathepsin Z, possesses exopeptidase activity and is thought to act by specific cleavage and thus activation of particular proteins, rather than by degradation of entire protein molecules [10, 11].

Cathepsin Z has been associated with the progression of, or the occurrence of metastasis in a number of cancers including non-small cell lung cancer [12], hepatocellular carcinoma [13] and melanoma [14]. Thus, the aim of the present experiments was to study the presence of the intracellular mature form of cathepsin Z in osteosarcoma cell lines, and to find out whether cathepsin Z protein affected cell activities in osteosarcoma cells associated with cancer progression. It was found that reduction of the cathepsin Z in osteosarcoma cells reduced their ability to invade through Matrigel.

Methods

Cell culture

Osteosarcoma cell lines, MG-63 [15] TE-85 [16,17] and SAOS-2 [18,19] with osteoblastic features were used for this study [6]. These cultured osteosarcoma cell lines and the control MCF-7, estrogen responsive breast cancer cell line [20], were grown in Dulbecco's modified Eagles' medium (DMEM) (Sigma, UK)

Table 1. SiRNA target sequences directed at cathepsin Z mRNA.

SiRNA	Target sequence	Qiagen Catalogue
SiRNA 1	5' CAGATACAACCTTGCCATCGA 3'	SI03064495
SiRNA 2	5' ATCGAGGAGCACTGTACATTT 3'	SI03048087
SiRNA 3	5' TCGGATCAACATCAAGAGGAA 3'	SI00025536
SiRNA 4	5' ACCGGAGGCATCTATGCCGAA 3'	SI00025529

supplemented with 10 % (v/v) foetal bovine serum (FBS), 2 % (v/v) 200 mM L-glutamine, 1 % (v/v) penicillin/streptomycin and 1X of DMEM Non-Essential Amino Acid Solution.

Isolation of RNA

Total RNA was extracted from cultured cells as described previously [21].

SiRNAs directed at cathepsin Z mRNA

Four small-interfering-RNA sequences (SiRNA), SiRNA 1-4, used for knockdown of cathepsin Z mRNA (GenBank accession NM_001336) and protein levels in MG-63 cells, were obtained from Qiagen (Table 1). Silencer negative control scrambled SiRNA (Ambion, Inc., USA) was used to check for any off-target effects.

Transient Transfection with SiRNA

SiRNAs were transiently transfected into cells using HiPerFect® Transfection Reagent (Qiagen, UK) according to the manufacturer's recommendations. Briefly, cells were seeded at 1×10^5 per well and grown for 24 h until cells reached 60% confluence. Culture medium without serum containing siRNA and HiPerFect® Transfection Reagent was added drop-wise onto the cells (to achieve a final concentration of 5 nM SiRNA) and the plate was gently swirled. Cells were incubated with normal DMEM medium containing 10% serum and the levels of cathepsin Z mRNA and protein were analyzed after 24 h.

Cell proliferation assay

Cell numbers were determined using a 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay [22]. Cells were plated into 96-well plates and allowed to grow for 6 days. Control wells with medium alone provided blanks for the absorbance readings. 50 µL of MTT stock solution was added to each well and incubated for 4 h. 200 µL of Dimethyl sulfoxide (DMSO) was added to each well, incubated for 10 min and the absorbance read at 570 nm in a SPECTROstar Nano plate reader.

Migration and invasion assays

Cells were seeded at 1×10^5 per well in 6-well plates and cultured until 80% confluent in normal DMEM cell culture medium. Scratches were made in the cell monolayers with a sterile 20 µL pipette tip and the cell monolayer was rinsed with Dulbecco's phosphate buffered saline (DPBS) and fresh DMEM medium added. The closure of the wound was monitored at 0 h and after 24 h intervals up to 3 days and observed using a Nikon Diaphot inverted microscope camera (D50). Analysis was carried out using ToupView software within the ToupTek package (Zhejiang, China). Image J software was used to measure the wound width in triplicate. The rates of wound closure in µm/hr from three separate experiments were then expressed as a percentage of the rates of untreated cells.

Chemotactic migration/invasive behaviour of the cells was assessed in a Transwell invasion assay using BD BioCoat™ Matrigel™ Invasion Chambers (BD Biosciences, USA). After rehydration of the Matrigel, 2.5×10^4 cells in serum-free medium (0.5 mL) were added into the upper chamber while DMEM medium with 10% serum (1 mL) was added in the lower chamber. For a negative control, serum-free medium was added to the lower chamber. The chambers were maintained at 37°C, 5% (v/v) CO_2 for 24 h. Using a cotton-wool swab, cells which had not invaded, were scrubbed from the membrane upper surface and washed off using DPBS. Kwik-Diff™ stain was used to visualize the cells on the lower membrane surface according to the supplier's protocol (Thermo Fisher, UK) and air-dried for at least 60 min. The number of invading cells was counted under a light microscope. The results are shown as the percentage of cells passing through the Matrigel membrane relative to untreated cells.

Western blotting

Cultured cells were washed twice with DPBS and lysed with 1 mL of cold radio immunoprecipitation assay (RIPA) buffer (ThermoFisher, UK). Protein concentrations were determined using a Thermo Scientific NanoDrop™ 2000 spectrophotometer and samples of extracts containing 20 µg of protein were separated by 12% (w/v) polyacrylamide Mini-PROTEAN® TGX™ precast protein gels (Bio-Rad laboratories, UK) and transferred to polyvinylidene difluoride membranes by electroblotting. The membranes were blocked with 5% (w/v) non-fat milk in Tris-buffered saline containing 0.1% (v/v) Tween 20 (TBST) for 1 h at room temperature and then incubated with the indicated primary antibodies overnight at 4°C, with continuous shaking. After washing three times with TBST, the membranes were incubated with the appropriate peroxidase-conjugated secondary antibody for 1 h at room temperature. Signals were detected using an Enhanced SuperSignal™ West Femto Chemiluminescent Substrate kit (Fisher Scientific, U.K.). The image data was collected using a Bio-Rad ChemiDoc digital imager (Bio-Rad, UK). The primary antibodies were as follows: anti-cathepsin Z, goat polyclonal, which recognizes both pro- and mature human cathepsin Z (amino acids 21-303 of cathepsin Z preproprotein, GenBank accession NP_001327) from R&D Systems, Cat. No AF934; anti human β-actin, mouse monoclonal from R&D Systems Cat. No. MAB8929 (both used at 1:10,000 dilution); anti estrogen receptor alpha, mouse monoclonal from R&D Systems, Cat. No. MAB57151 (used at 1:20,000). Secondary antibodies were peroxidase-conjugated donkey anti-goat IgG for cathepsin Z and sheep anti-mouse IgG for β-actin and estrogen receptor alpha (both at 1:10,000; Fisher Scientific, U.K.).

Statistical analysis

Statistical analyses using Stats Direct 3 software (Altrincham, Cheshire) were carried out as described previously [21].

Results

Cathepsin Z protein is present in estrogen receptor α positive osteosarcoma cells

The presence of cathepsin Z protein in three osteosarcoma cell lines, MG-63, TE-85 and SAOS-2, were examined by Western

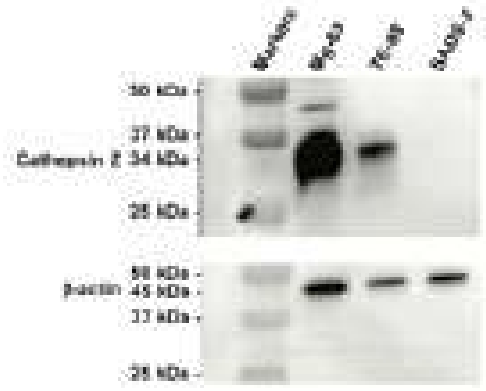

Figure 1. Cathepsin Z protein is present in the osteosarcoma cell line, MG-63, by Western blotting. Three cultured osteosarcoma cell lines, MG-63, TE85 and SAOS-2, were grown as described in Materials and Methods and cell extracts were subjected to Western blotting with chemiluminescence detection using antibodies to cathepsin Z and β-actin.

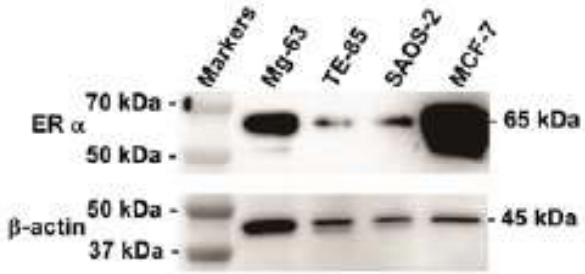

Figure 2. Estrogen receptor α in osteosarcoma cell lines, MG-63, TE-85, SAOS-2 and in the breast cancer cell line MCF-7, by Western blotting. Cell lines were grown as described in Materials and Methods and cell extracts were subjected to Western blotting with chemiluminescence detection using antibodies to estrogen receptor α (ER α) and β-actin.

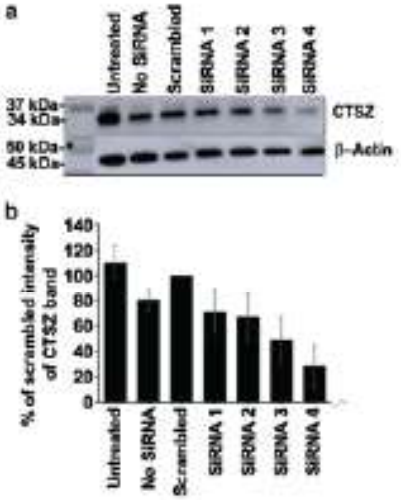

Figure 3. The effect of knock-down of cathepsin Z protein in cultured MG-63 osteosarcoma cells. MG-63 osteosarcoma cells were transfected with one of four siRNAs (SiRNA 1, SiRNA 2, SiRNA 3, SiRNA 4) or with a control, scrambled SiRNA (scrambled) or with transfection reagents only (No SiRNA) or left untreated (untreated) as described in Methods. After 24 hours, the levels of cathepsin Z protein and β-actin were determined by Western blotting in cell extracts (panel a) and the resulting bands quantified (panel b) relative to those in the scrambled control, as described in Methods. Error bars represent SD of three separate experiments.

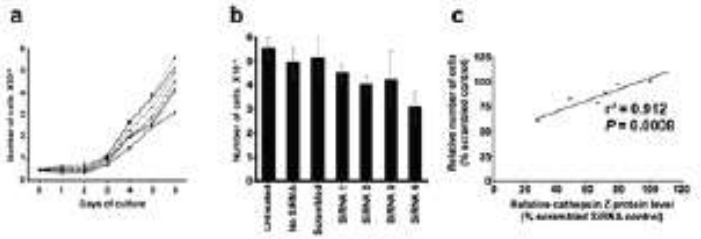

Figure 4. The effect of knock-down of cathepsin Z protein on the accumulation of MG-63 osteosarcoma cells in culture. MG-63 osteosarcoma cells were transfected with one of four siRNAs (SiRNA 1, SiRNA 2, SiRNA 3, SiRNA 4) or with a control, scrambled SiRNA (scrambled) or with transfection reagents only (No SiRNA) or left untreated (untreated), as described in Materials and Methods. The number of cells in the various treated and untreated cultures was determined daily for 6 days (Panel a) ···■···Untreated; -•-, No SiRNA; —●—, Scrambled; —▲—, SiRNA 1; --•--, SiRNA 2; —|—, SiRNA 3; —■—, SiRNA 4. The mean number of cells present after 6 days of culture (panel b) was determined ± SD in three separate experiments. Panel c, the relationship between the relative cathepsin Z protein levels and the number of cells after 6 days of culture.

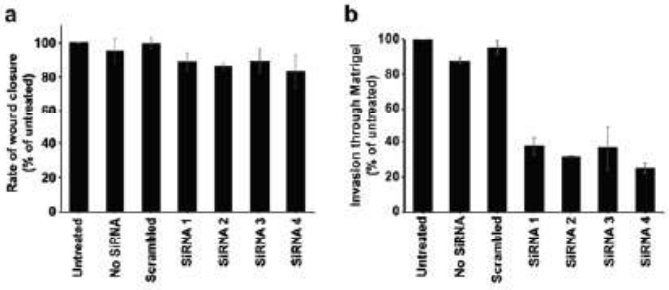

Figure 5. The effect of knock-down of cathepsin Z protein on the migration and invasion on MG-63 osteosarcoma cells in culture. The MG-63 cells were subjected to scratch wound migration assays on plastic as described in Materials and Methods and the rates of wound closure were normalised to those of the untreated cells (panel a). Transwell chemotactic migration/invasion assays though a Matrigel membrane were carried out as described in Materials and Methods. The numbers of cells passing through the Matrigel and 8 μm pores in the supporting membrane were counted and the mean numbers ± SD of three experiments were expressed as a proportion of the number of untreated cells passing through the membrane and filter (panel b).

blotting. The mature, 34 kDa cathepsin Z protein was found to be abundantly present in the MG-63 cells, present, but at a lower level, in the TE-85 cells and below the level of detection in the SAOS-2 cells (Figure 1). There was an additional band with a molecular weight corresponding to the 40 kDa pro-cathepsin Z protein. However, this was only a minor band compared to that of the mature, 34 kDa cathepsin band.

In order to find out the relationship between cathepsin Z and the presence of estrogen receptor, the expression of estrogen receptor α was determined in the MG-63, TE-85, SAOS-2 cells and in the control, estrogen-receptor-positive breast-cancer-derived cell line, MCF-7 by Western blotting. Estrogen receptor α protein was abundantly present as a 65 kDa band [23] in the MG-63 and the control MCF-7 cells, but present at lower levels in the TE-85 and SAOS-2 cells (Figure 2). The level of cathepsin Z thus broadly follows the level of estrogen receptor α in these cells.

SiRNAs reduces the expression of cathepsin Z protein in cultured osteosarcoma cells, MG-63

In order to identify cell activities associated with the expression of cathepsin Z in MG-63 cells, cathepsin Z protein levels were experimentally knocked down using SiRNA. Four separate cathepsin Z-mRNA-targeting SiRNAs, designated 1-4 (Table 1), individually significantly reduced the level of the 34 kDa band of mature cathepsin Z protein after 24 h by 29.2, 33.8, 51.5 and 72.1%, respectively (Figure 3a and 3b), compared to cells transfected with a scrambled control SiRNA (SiRNAs 1-4, P = 0.039, 0.015, 0.0003 and <0.0001, respectively, Analysis of variance (ANOVA) with Dunnett's post hoc test). The results show that SiRNA 4 exhibited the largest reduction of cathepsin Z protein in the MG-63 cells (72% knockdown), when compared to cells transfected with a control scrambled SiRNA.

The effect of knockdown of cathepsin Z using SiRNAs on the growth of MG-63 osteosarcoma cells

The effects of the SiRNAs on the growth of the MG-63 cells were examined. All SiRNAs reduced the growth of the MG-63 cell line, with SiRNA 4 reducing the number of MG-63 cells, as indicated using the MTT assay, by 40% after 6 days (Figure 4a and 4b). There was a strongly significant correlation between the reduction in growth rate compared to scrambled SiRNA controls and the degree of knockdown of the cathepsin Z protein (correlation coefficient, r = 0.955, r2 = 0.912, P = 0.0008; Figure 4c).

The effect of knock down of cathepsin Z protein by siRNAs in MG-63 osteosarcoma cells is associated predominantly with cell invasion, but not with cell migration

Knockdown of cathepsin Z protein had only a small effect on cell migration in the MG-63 cells, as measured in an in vitro scratch wound assay (Figure 5a). Untreated cells and cells treated with scrambled SiRNA showed virtually the same rates of wound closure. Compared to cells treated with scrambled SiRNA controls, cells treated with transfection reagent alone (No SiRNA) exhibited a small, but insignificant, 4.4% reduction in the rate of wound closure (P = 0.852, ANOVA with Dunnett's post hoc test). Cells treated with SiRNAs 1 and 3 exhibited non-significant reductions in the rate of wound closure of 10.8%, and 10.4% (P = 0.16 and 0.18, respectively, ANOVA with Dunnett's post-hoc test). Cells treated with SiRNAs 2 and 4, which yielded the highest reduction in growth rate, exhibited small, but significant, reductions of only 13.8% and 16.8% in the rate of wound closure, compared to cells treated with scrambled SiRNA controls (P = 0.05 and 0.015, respectively, ANOVA with Dunnett's post-hoc test).

In contrast to the scratch migration results, reduction in the level of cathepsin Z protein caused a much greater reduction in the chemotactic migration/invasion of cells through Matrigel in a Transwell assay system than in the scratch migration assay. MG-63 cells treated with transfection reagents alone with no SiRNA (No SiRNA) or transfected with scrambled SiRNA exhibited invasive potential of 88% and 95% of untreated cells (Figure 5b), respectively, that were not significantly different from scrambled controls (P = 0.14 and 0.8, average of two independent experiments). However, compared to the scrambled SiRNA controls, SiRNAs 1-4, individually, significantly reduced the invasive ability of MG-63 cells by 61, 67, 62 and 74%, respectively (all P = < 0.0001, ANOVA with Dunnett's post hoc test). The reduction in invasive potential in 24h was not likely due to the 40% inhibitory effect on cell numbers, which occurred over the longer period of 6 days (Figure 4a).

Discussion

In the present experiments, the mature form of cathepsin Z protein has been shown to be abundantly expressed in extracts of the osteosarcoma cell line, MG-63, in culture as a 34 kDa band. This band is unlikely to be due to cross reaction with either the 38 kDa pro-form [24], or the 30/31 kDa mature form [25,26] of the closely-related cathepsin B, which is also present in the MG-63 cells [27].

The observed abundant expression of cathepsin Z protein in the MG-63 cells is consistent with the abundant level of mRNA signal by Reverse transcription polymerase chain reaction (RT-PCR) reported previously [7]. In the present experiments, the high level of the mature form of cathepsin Z appears to contrast with the predominantly pro-form found previously in MG-63 cells [7]. However, in the present experiments, whole cell extracts were used, whereas in the previous experiments [7], either cell membrane preparations or secreted protein in the culture medium were detected.

Cathepsin Z was detected at a lower level in the TE-85 and not detected in the SAOS-2, osteosarcoma cell lines. It has been suggested that the TE-85 and SAOS-2 cells represent sequentially later stages of osteoblast development than the MG-63 cells [5,6]. The declining occurrence of cathepsin Z protein in the order MG-63, TE-85 and SAOS-2 suggests an association of cathepsin Z protein with the less differentiated osteosarcoma cells. However, the MG-63 cells also contained higher levels of estrogen receptor α than the TE-85 or SAOS-2 cells, but lower levels than in the estrogen receptor α positive MCF-7, a result found previously [28], thus it is possible that cathepsin Z expression is dependent upon the presence of estrogen receptor in these osteosarcoma cells. Estrogen induction of cathepsin Z has been reported previously in an experimental system of estrogen-receptor-α-transfected human foetal osteoblast cells [29], reinforcing the association of cathepsin Z with the presence of estrogen receptor.

Although there were small inhibitory effects of knockdown of cathepsin Z on cell growth rate and scratch migration, the predominant effect of the experimental reduction of cathepsin Z mRNA and protein levels in the MG-63 cell line with SiRNA, was a 60-75% reduction in the ability of the osteosarcoma cells to invade through Matrigel after just 24 h. The results presented here, suggest, for the first time, a role for cathepsin Z in osteosarcoma cell chemotactic migration/invasion. Since many osteosarcoma tumours from patients are estrogen-receptor negative [30], it will be important to establish whether cathepsin Z protein is expressed in human osteosarcomas independently of estrogen receptor and thereby contributes to their invasion and metastasis. The presence of cathepsin Z in TE-85 cells, which contain only low levels of estrogen receptor α, suggests that this might be the case. Considering the complexity of bone cancer processes, it will be necessary to investigate cathepsin Z involvement in osteosarcoma development using in vivo and in vitro model systems in order to fully understand the involvement and molecular mechanisms of cathepsin Z associated with osteosarcoma development.

Conclusion

This study showed that cathepsin Z was present abundantly in estrogen receptor a positive osteosarcoma cells, MG-63 and was also present in TE-85 cells, but below the level of detection in SAOS-2 cells. Knock-down of cathepsin Z protein by specifically targeted siRNAs in the MG-63 cells had only a small effect on cell growth and scratch migration, but dramatically reduced the chemotactic migration/invasion of these cells through a Matrigel matrix. This is the first time that cathepsin Z has been shown to

play a causative role in osteosarcoma chemotactic cell migration/invasion and suggests that cathepsin Z might contribute to osteosarcoma progression. Further study using in vivo and in vitro model systems of osteosarcoma will be necessary to understand the molecular mechanisms of cathepsin Z associated with osteosarcoma development.

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References

1. Miller BJ, Cram P, Lynch CF, Buckwalter JA. Risk factors for metastatic disease at presentation with osteosarcoma: an analysis of the SEER database. *J Bone Joint Surg Am*. 2013; 95: e89.
2. Smeland S, Bielack SS, Whelan J, Bernstein M, Hogendoorn P, et al. Survival and prognosis with osteosarcoma: outcomes in more than 2000 patients in the EURAMOS-1 (European and American Osteosarcoma Study) cohort. *Eur J Cancer.* 2019; 109: 36-50.
3. Imura Y, Takenaka S, Kakunaga S, Nakai T, Wakamatsu T, et al. Survival analysis of elderly patients with osteosarcoma. *Int Orthop.* 2019; 43: 1741-1747.
4. Mutsaers AJ, Walkley CR. Cells of origin in osteosarcoma: mesenchymal stem cells or osteoblast committed cells? *Bone*. 2014; 62: 56-63.
5. Clover J, Gowen M. Are MG-63 and HOS TE85 human osteosarcoma cell lines representative models of the osteoblastic phenotype? *Bone*. 1994; 15: 585-591.
6. Pacheco-Pantoja EL, Ranganath LR, Gallagher JA, Wilson PJ, Fraser WD. Receptors and effects of gut hormones in three osteoblastic cell lines. *BMC Physiol*. 2011; 11: 12.
7. Staudt ND, Aicher WK, Kalbacher H, Stevanovic S, Carmona AK, et al. Cathepsin X is secreted by human osteoblasts, digests CXCL-12 and impairs adhesion of hematopoietic stem and progenitor cells to osteoblasts. *Haematologica.* 2010; 95: 1452-1460.
8. Klemencic I, Carmona AK, Cezari MH, Juliano MA, Juliano L, et al. Biochemical characterization of human cathepsin X revealed that the enzyme is an exopeptidase, acting as carboxymonopeptidase or carboxydipeptidase. *Eur J Biochem.* 2000; 267: 5404-5412.
9. Lechner AM, Assfalg-Machleidt I, Zahler S, Stoeckelhuber M, Machleidt W, et al. RGD-dependent binding of procathepsin X to integrin alphavbeta3 mediates cell-adhesive properties. *J Biol Chem*. 2006; 281(: 39588-39597.
10. Pecar Fonovic U, Kos J. Cathepsin X Cleaves Profilin 1 C-Terminal Tyr139 and Influences Clathrin-Mediated Endocytosis. *PLoS One.* 2015;10: e0137217.
11. Staudt ND, Maurer A, Spring B, Kalbacher H, Aicher WK, et al. Processing of CXCL12 by different osteoblast-secreted cathepsins. *Stem Cells Dev.* 2012; 21: 1924-1935.
12. Li W, Yu X, Ma X, Xie L, Xia Z, et al. Deguelin attenuates non-small cell lung cancer cell metastasis through inhibiting the CtsZ/FAK signaling pathway. *Cell Signal.* 2018; 50: 131-141.
13. Wang J, Chen L, Li Y, Guan XY. Overexpression of cathepsin Z contributes to tumor metastasis by inducing epithelial-mesenchymal transition in hepatocellular carcinoma. *PLoS One.* 2011; 6: e24967.
14. Rumpler G, Becker B, Hafner C, McClelland M, Stolz W, et al. Identification of differentially expressed genes in models of melanoma progression by cDNA array analysis: SPARC, MIF and a novel cathepsin protease characterize aggressive phenotypes. *Exp Dermatol.* 2003; 12: 761-771.
15. Billiau A, Edy VG, Heremans H, Van Damme J, Desmyter J, et al. Human interferon: mass production in a newly established cell line, MG-63. *Antimicrob Agents Chemother.* 1977: 12: 11-15.
16. McAllister RM, Filbert JE, Nicolson MO, Rongey RW, Gardner MB, et al. Transformation and productive infection of human osteosarcoma cells by a feline sarcoma virus. *Nat New Biol.* 1971; 230: 279-282.
17. McAllister RM, Gardner MB, Greene AE, Bradt C, Nichols WW, et al. Cultivation in vitro of cells derived from a human osteosarcoma. *Cancer.* 1971; 27: 397-402.
18. Murray E, Provvedini D, Curran D, Catherwood B, Sussman H, et al. Characterization of a human osteoblastic osteosarcoma cell line (SAOS-2) with high bone alkaline phosphatase activity. *J Bone Miner Res.* 1987; 2: 231-238.
19. Rodan SB, Imai Y, Thiede MA, Wesolowski G, Thompson D, et al. Characterization of a human osteosarcoma cell line (Saos-2) with osteoblastic properties. *Cancer Res.* 1987; 47: 4961-4966.
20. Liu D, Rudland PS, Sibson DR, Platt-Higgins A, Barraclough R. Human homologue of cement gland protein, a novel metastasis inducer associated with breast carcinomas. *Cancer Res.* 2005; 65: 3796-3805.
21. Dera AA, Ranganath L, Barraclough R, Vinjamuri S, Hamill S, et al. Cathepsin Z as a novel potential biomarker for osteoporosis. *Sci Rep*. 2019; 9: 9752.
22. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods.* 1983; 65: 55-63.
23. Luo XH, Liao EY. Effects of estriol on the proliferation and differentiation of human osteoblastic MG-63 cells. *Endocr Res.* 2003; 29: 343-351.
24. Cavallo-Medved D, Moin K, Sloane B. Cathepsin B: Basis Sequence: Mouse. AFCS Nat Mol Pages. 2011.
25. Mort JS, Buttle DJ. Cathepsin B. *Int J Biochem Cell Biol.* 1997; 29: 715-720.
26. Mitrovic A, Pecar Fonovic U, Kos J:. Cysteine cathepsins B and X promote epithelial-mesenchymal transition of tumor cells. *Eur J Cell Biol.* 2017; 96: 622-631.
27. Aisa MC, Rahman S, Senin U, Maggio D, Russell RG. Cathepsin B activity in normal human osteoblast-like cells and human osteoblastic osteosarcoma cells (MG-63): regulation by interleukin-1 beta and parathyroid hormone. *Biochim Biophys Acta.* 1996; 1290: 29-36.
28. Dohi O, Hatori M, Suzuki T, Ono K, Hosaka M, Akahira J, Miki Y, Nagasaki S, Itoi E, Sasano H: Sex steroid receptors expression and hormone-induced cell proliferation in human osteosarcoma. *Cancer Sci.* 2008; 99: 518-523.
29. Liu M, Goss PE, Ingle JN, Kubo M, Furukawa Y, Batzler A, Jenkins GD, Carlson EE, Nakamura Y, Schaid DJ et al: Aromatase inhibitor-associated bone fractures: a case-cohort GWAS and functional genomics. *Mol Endocrinol.* 2014; 28: 1740-1751.
30. Lillo Osuna MA, Garcia-Lopez J, El Ayachi I, Fatima I, Khalid AB, Kumpati J, Slayden AV, Seagroves TN, Miranda-Carboni GA, Krum SA: Activation of Estrogen Receptor Alpha by Decitabine Inhibits Osteosarcoma Growth and Metastasis. *Cancer Res.* 2019; 79: 1054-1068.

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